NTP
National Toxicology Program

DNA Based Therapies

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FDA Nomination of DNA Based Therapies

- Limited authority to require non-acute long term studies for biologicals
- Majority of sponsors are small biotechnology companies or academic institutions that lack the resources to support extensive preclinical studies
- Non dissemination of proprietary information making regulatory consensus difficult
- DNA based therapies are the fastest growing segment of the product portfolio of the FDA Center for Biologic Evaluation and Research (CBER)

DNA Based Therapies

- Viral Vectors – gene therapy
- Bacterial Plasmids – plasmid based vaccines
- Non Coding RNA – siRNA, ribozymes
- Synthetic Oligonucleotides – immuno adjuvants

Current Activities

- Joint studies with FDA to examine insertional mutagenesis of retroviral and lentiviral vectors in hematopoietic stem cells

- Collaborative studies with NIDCR to evaluate the safety of using the protein secreting ability of salivary glands transduced with different vector-transgene combinations, to express and secrete transgene encoded therapeutic proteins into serum for treatment of single protein deficiency diseases

Insertional Mutagenesis Associated with Integration of Retroviral Vectors

- Integration occurs at random (?) genomic locations within transcriptionally active chromatin
- Portions of viral genome that remain in the vector (LTR) may contain promotor/enhancer sequences or other sequences that disrupt control of cellular genes
- Integration may disrupt the coding or control region of a gene
- The ectopic expression of the transgene product may alter or disrupt host cell signaling leading to activation of an oncogene or inactivation of a tumor supressor gene.

Ex-vivo Exposure with Retroviral Vectors

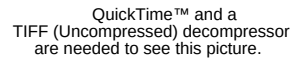
- Use vector to introduce (integrate into genome) therapeutic gene into population of stem cells: repopulating hematopoietic stem cells (HSC's)
- Serve as renewable source of cells expressing therapeutic gene product, providing permanent correction

- bone marrow; enrich for HSC
- ex-vivo exposure to vector
- ex-vivo expansion
- reinfusion

X-linked SCID

- SCID: severe-combined-immunodeficiency-syndrome
- Characterized by severe lymphopenia at birth; uniformly fatal within two years without intervention
- Associated with mutations in 9 genes that exhibit either x-linked recessive or autosomal recessive pattern of inheritance
- X-linked SCID is the most common form
- Caused by mutations in the gene encoding the common gamma chain (γ_c) a component of cell surface receptors for several interleukins (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21)

First Successful Human Gene Therapy Trial



Cavazzana-Calvo et al Science 288, 2000

Insertional Mutagenesis in a Human Clinical Trial Involving a Retroviral Vector

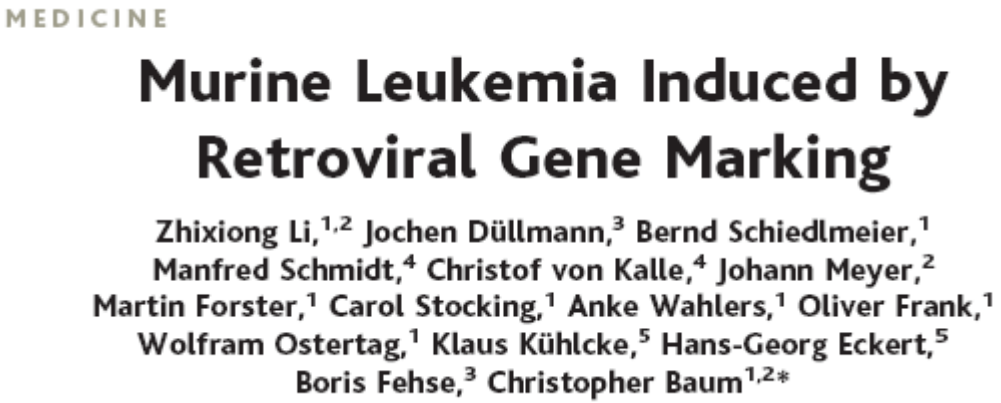
LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hacein-Bey-Abina,[1,2*] C. Von Kalle,[6,7,8] M. Schmidt,[6,7]
M. P. McCormack,[9] N. Wulffraat,[10] P. Leboulch,[11] A. Lim,[12]
C. S. Osborne,[13] R. Pawliuk,[11] E. Morillon,[2] R. Sorensen,[19]
A. Forster,[9] P. Fraser,[13] J. I. Cohen,[15] G. de Saint Basile,[1]
I. Alexander,[16] U. Wintergerst,[17] T. Frebourg,[18] A. Aurias,[19]
D. Stoppa-Lyonnet,[20] S. Romana,[3] I. Radford-Weiss,[3] F. Gross,[2]
F. Valensi,[4] E. Delabesse,[4] E. Macintyre,[4] F. Sigaux,[20] J. Soulier,[21]
L. E. Leiva,[14] M. Wissler,[6,7] C. Prinz,[6,7] T. H. Rabbitts,[9]
F. Le Deist,[1] A. Fischer,[1,5†‡] M. Cavazzana-Calvo[1,2†]

We have previously shown correction of X-linked severe combined immunodeficiency [SCID-X1, also known as γ chain (γc) deficiency] in 9 out of 10 patients by retrovirus-mediated γc gene transfer into autologous CD34 bone marrow cells. However, almost 3 years after gene therapy, uncontrolled exponential clonal proliferation of mature T cells (with γδ+ or αβ+ T cell receptors) has occurred in the two youngest patients. Both patients' clones showed retrovirus vector integration in proximity to the *LMO2* proto-oncogene promoter, leading to aberrant transcription and expression of *LMO2*. Thus, retrovirus vector insertion can trigger deregulated premalignant cell proliferation with unexpected frequency, most likely driven by retrovirus enhancer activity on the *LMO2* gene promoter.

Hacein-Bay Abina et al, Science 302, 2003

Insertional Mutagenesis in Mouse Gene Marking Study



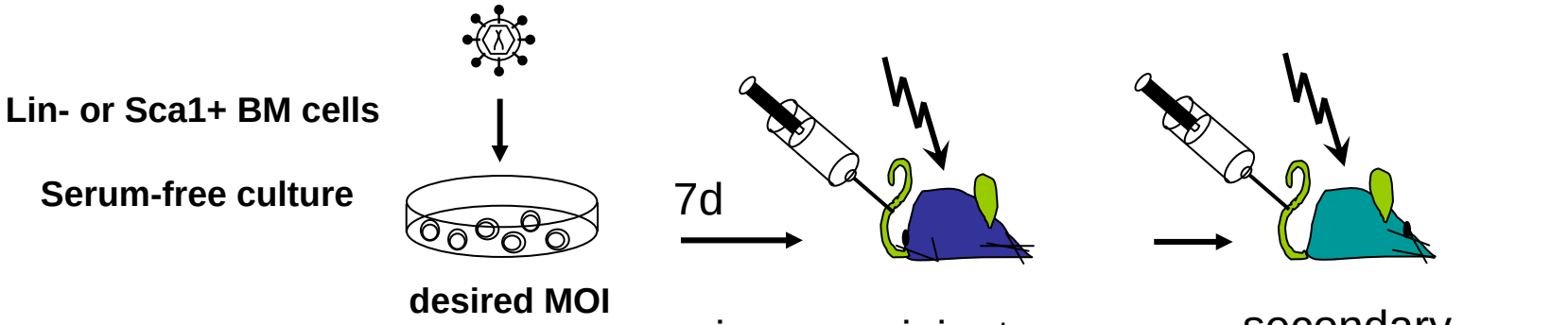
MEDICINE

Murine Leukemia Induced by Retroviral Gene Marking

Zhixiong Li,[1,2] Jochen Düllmann,[3] Bernd Schiedlmeier,[1] Manfred Schmidt,[4] Christof von Kalle,[4] Johann Meyer,[2] Martin Forster,[1] Carol Stocking,[1] Anke Wahlers,[1] Oliver Frank,[1] Wolfram Ostertag,[1] Klaus Kühlcke,[5] Hans-Georg Eckert,[5] Boris Fehse,[3] Christopher Baum[1,2]*

Li et al, Science 296, 2002

Serial Bone Marrow Transplantation Model

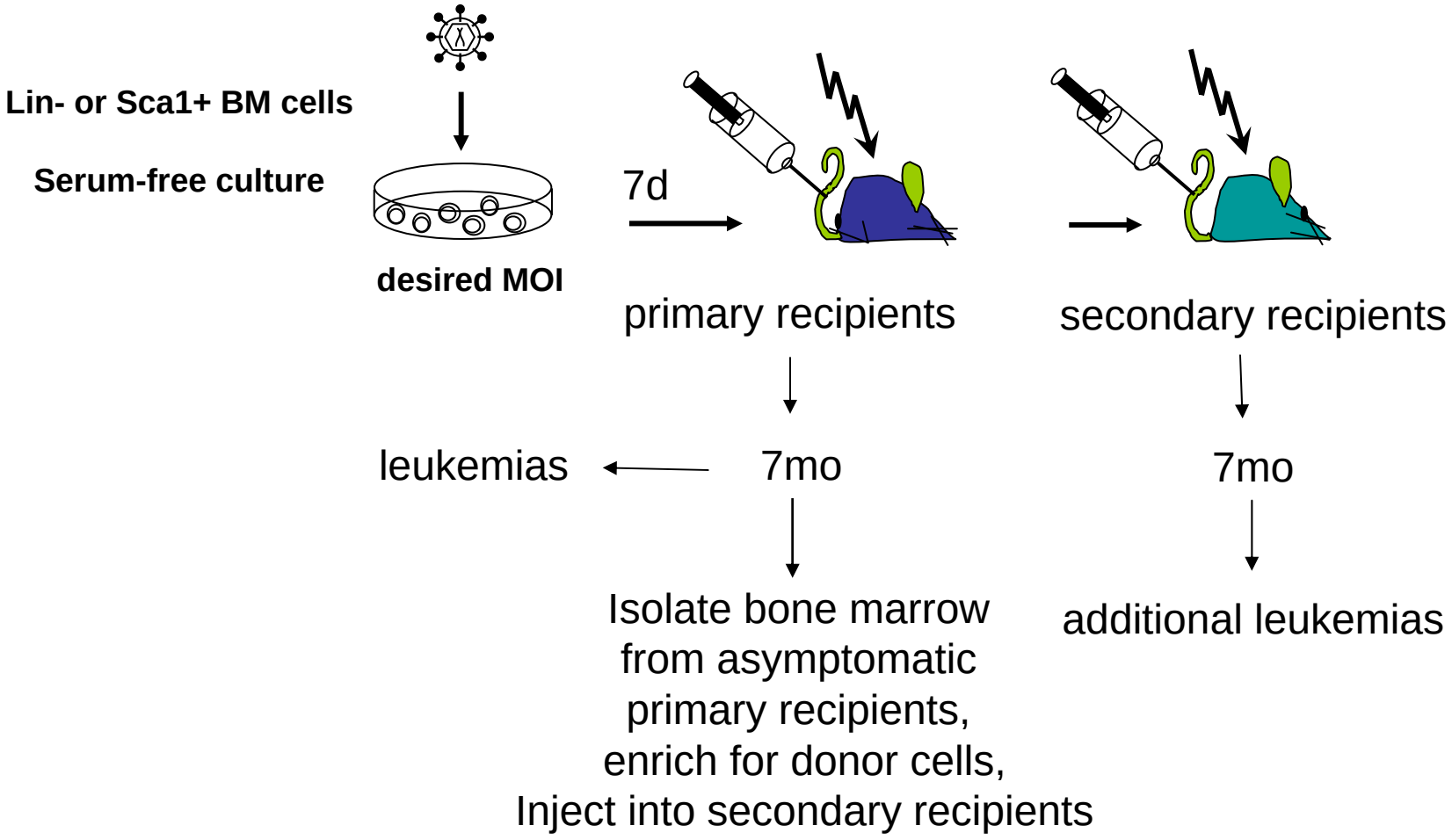


Li et al, Science 296, 2002

Factors Influencing Retroviral Vector Mediated Insertional Mutagenesis leading to Tumor Development

- Vector Architecture
  - backbone elements
  - transgene
  - presence or absence of certain promotor- enhancer elements
- Dose - multiplicity of insertions
- Insertion sites

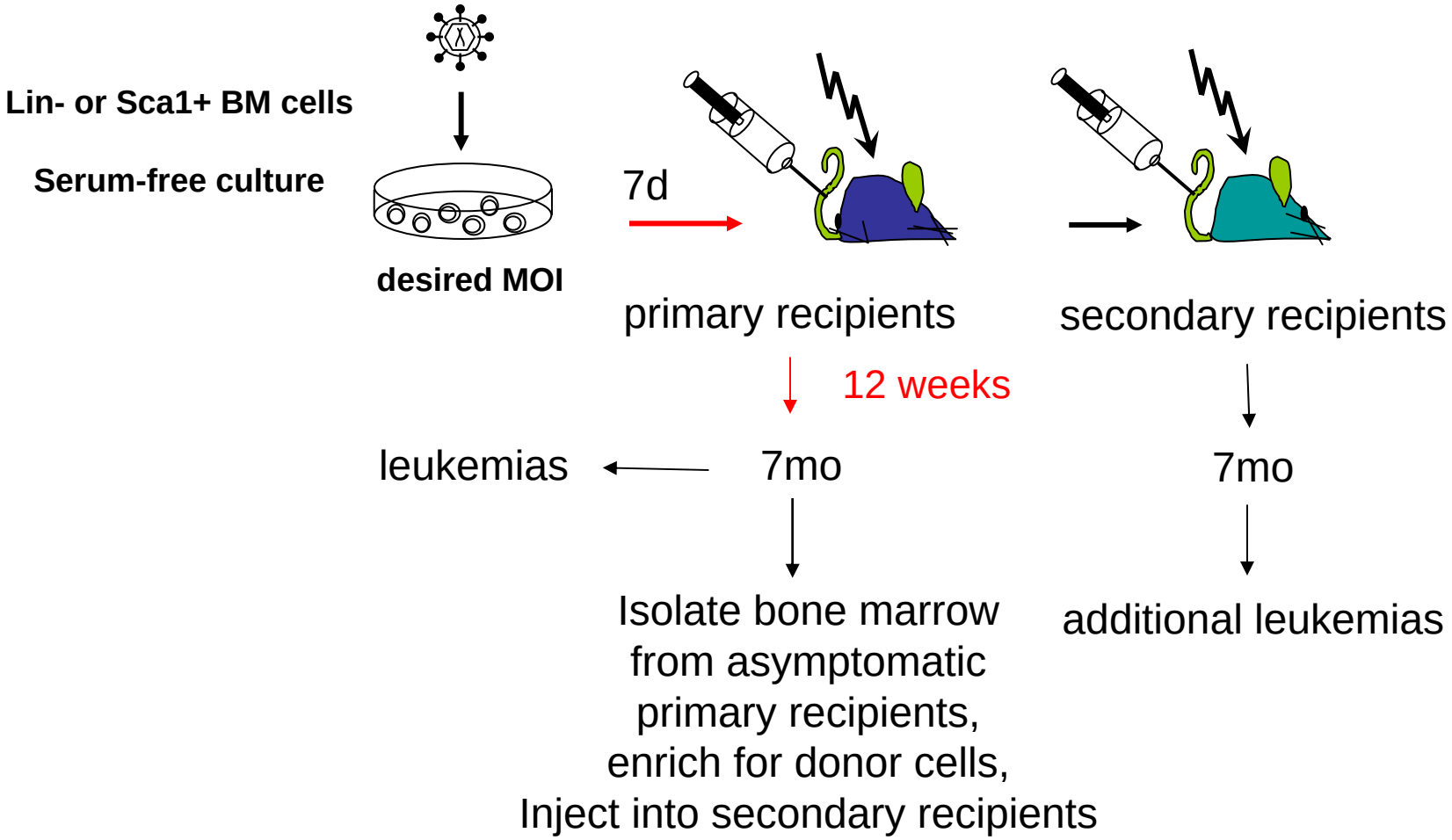
Assay for Insertional Mutagenesis



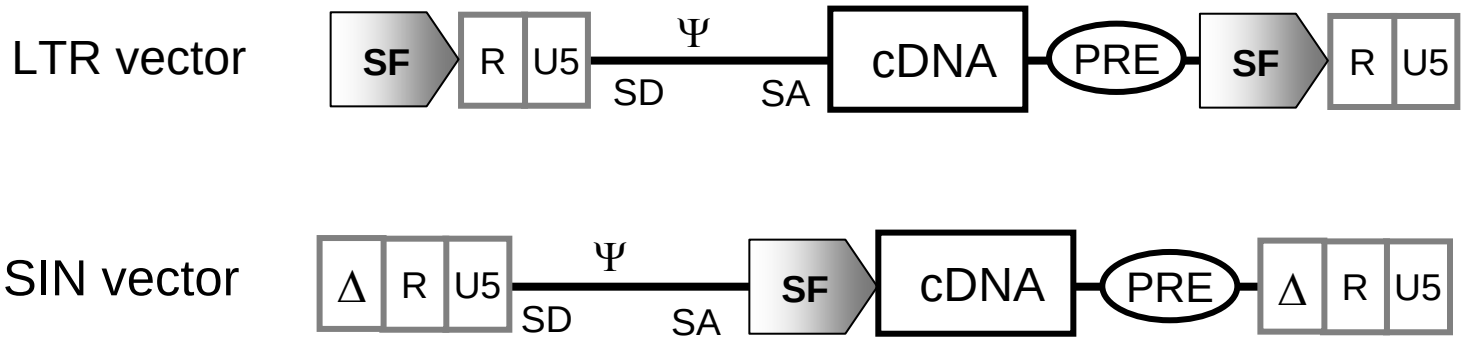
Animal Models

- Donors: female C57BL/6
  - Carry the normal *Ptprcb* allele
- Acceptors: female B6.SJL - *Ptprca Pepcb* /BoyJ
  - *Ptprca* and *Pepcb* are closely linked alleles serially backcrossed from SJL/J onto C57BL/6
- Ptpr = protein tyrosine phosphatase receptor; cell surface protein aka CD45 allows separation of donor (*b* allele) and acceptor (*a* allele) bone marrow cells

Pilot Study


Lin- or Sca1+ BM cells
Serum-free culture
desired MOI
7d
primary recipients
secondary recipients
12 weeks
leukemias
7mo
7mo
Isolate bone marrow
from asymptomatic
primary recipients,
enrich for donor cells,
Inject into secondary recipients
additional leukemias

Vector Design for Initial FDA Study



Transgene: enhanced green fluorescent protein

EGFP

Flow cytometry results for bone marrow

- Robust (>50 %) donor engraftment observed in:
  - 10/15 NTPMOCK
  - 15/15 NTPLTR
  - 8/9 NTPSIN
- NTPLTR mice had greater donor cell engraftment and higher total % EGFP+ cells relative to NTPSIN

Definitive Studies

- 50 animals per group; 2 multiplicities of infection (MOI), low and high;
- After 7 months bone marrow from each vector exposed asymptomatic primary recipient will be transplanted into 2 irradiated secondary recipients and maintained for another 7 months;
- Monthly transgene analysis and leukemia monitoring in peripheral blood;
- collection of tissues for DNA extraction and insertion site mapping

Gene Transfer to Rat and Mouse Salivary Glands: Dr. Bruce Baum, NIDCR

- Investigate the feasibility and safety of using the protein secreting ability of salivary glands transduced with different vector-transgene combinations, to express and secrete transgene encoded therapeutic proteins;
- Salivary glands are capable of endocrine secretion of proteins directly into serum;
- Treatment of single protein deficiency diseases:
  - Growth hormone
  - Clotting factors
- Treatment of salivary gland disorders: loss of saliva secretion associated with radiation treatment of head and neck cancers

Advantages of Salivary Gland Transduction

- In vivo transduction - direct access to salivary glands through cannulation of excretory ducts; no surgery required
- In humans, cannulation is a routine clinical procedure performed without anesthesia
- Human salivary glands are encapsulated, potentially limiting the systemic dissemination of gene transfer vectors
- Salivary glands can be surgically removed in case of complications

Major Objectives

- Evaluate toxicity associated with salivary gland transduction
- Persistence and duration of transgene expression
- Distribution of therapeutic protein between serum and saliva
- Biological response to transgene encoded protein
- Systemic distribution of vector
- Effect of vector dose on transduction efficiency

Typical Study Protocol

- Day 1: animals (males and females, 5-10/group) cannulated in right submandubular gland duct (under anesthesia) and solution of vector in saline injected via syringe connected to cannula
- Typically 4-5 dose groups (vector particles/unit volume)
- Standard in life monitoring
- Days 3, 29, 57, 92 collect blood and saliva, necropsy animals and collect tissues
- Hematology, clinical chemistry, histopathology
- QPRC on testis/ovary, spleen, liver, lungs, heart, right kidney, intestine, brain, tongue, peripheral blood, saliva, draining lymph node, right and left submandibular gland

Vector Transgene Combinations

- AdCMVhGH: adenoviral vector with human growth hormone gene driven from a CMV promotor
- No effect on food or water consumption
- Dose related levels of hGH in serum
- Vector present primarily in submandibular glands

Vector Transgene Combinations

- AdhAQP1 adenoviral vector with human aquaporin-1 transgene
- No adverse effects; vector localized to salivary glands
- Previous studies in minipigs and rats demonstrated that transduction of salivary glands in animals that were irradiated in the head/neck region restored lost salivary gland function
- Currently this vector is being used in a human clinical trial to determine if this treatment may be used in humans that have lost salivary gland function as a result of irradiation for head and neck cancers

Vector Transgene Combinations

- AAVhEPO: vector based on adeno associated virus carrying human erythropoietin transgene hEPO
- No adverse toxic effects
- Dose related increase in erythropoiesis
- Biodistribution revealed significant differences between males and females

Studies Completed

- Voutetakis A, Zheng C, Wang J, Goldsmith CM, Afione S, Chiorini JA, Wenk ML, Vallant M, Irwin RD, Baum BJ. Gender differences in serotype 2 adeno-associated virus biodistribution after administration to rodent salivary glands.
  Hum Gene Ther. 2007 Nov;18(11):1109-18.

- Zheng C, Goldsmith CM, Mineshiba F, Chiorini JA, Kerr A, Wenk ML, Vallant M, Irwin RD, Baum BJ. Toxicity and biodistribution of a first-generation recombinant adenoviral vector, encoding aquaporin-1, after retroductal delivery to a single rat submandibular gland.
  Hum Gene Ther. 2006 Nov;17(11):1122-33.

- Zheng C, Voutetakis A, Kok MR, Goldsmith CM, Smith GB, Elmore S, Nyska A, Vallant M, Irwin RD, Baum BJ. Toxicity and biodistribution of a first-generation recombinant adenoviral vector, in the presence of hydroxychloroquine, following retroductal delivery to a single rat submandibular gland.
  Oral Dis. 2006 Mar;12(2):137-44.

Summary

- Development and validation of models for preclinical evaluation of viral vectors and other DNA containing therapies
- Data from studies conducted by the NTP is readily available in the public domain and will serve as a resource for regulators and researchers

Collaborators

FDA Division of Cellular and Gene Therapy
- Carolyn Wilson
- Mercedes Serabian

Cincinnati Childrens Medical Center
- Lilith Reeves
- Chris Baum

Battelle Laboratories
- Milton Hejtmancik
- Laurie Fomby

NIDCR
- Bruce Baum
- Changyu Zheng

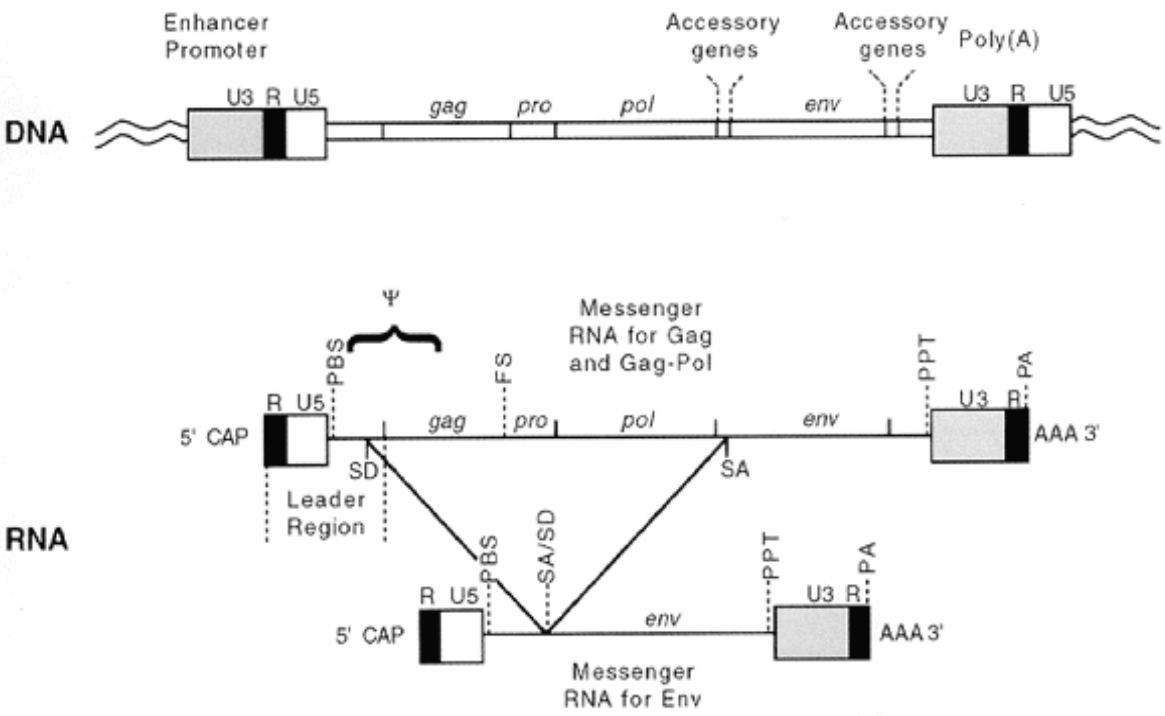
Bioreliance
- Marty Wenk

NTP
- Molly Vallant

PCR

- qPRC
  - Marking primarily present in the bone marrow and spleen of NTP-LTR and NTP-SIN
  - Vector was only detected in peripheral blood of 5 animals
- ecoRCR
  - No amplification for viral ecotropic envelope sequences
- LM-PCR
  - No monoclonal dominance noted
  - All NTPLTR and NTPSIN animals demonstrated vector insertions

Genetic Organization of Simple Retrovirus



Construction of a Retroviral Vector

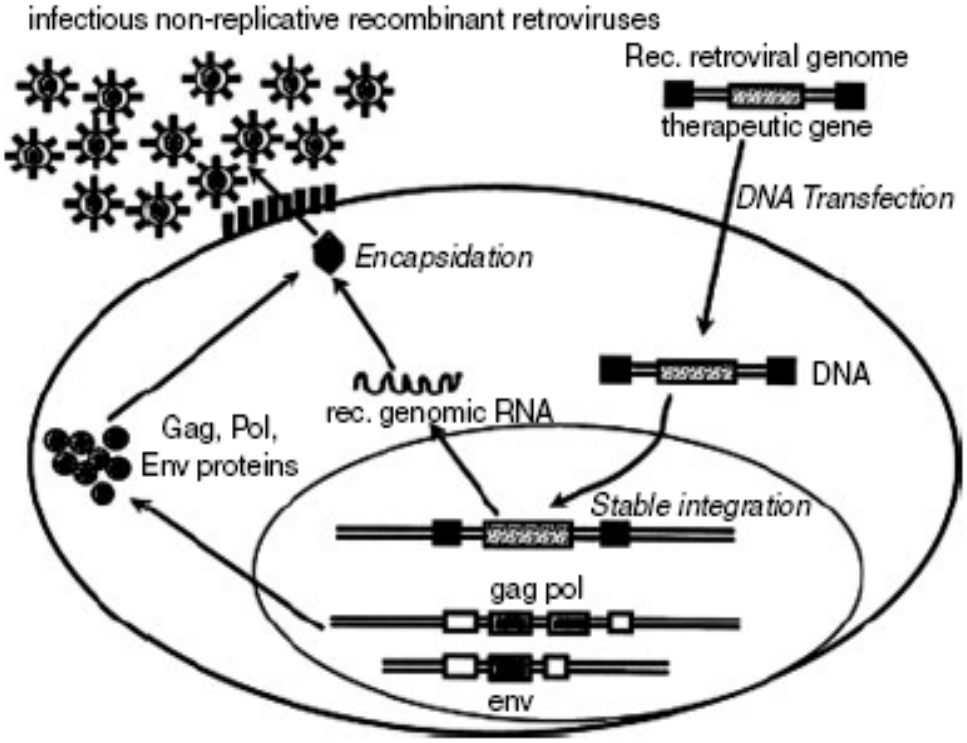


Figure 1. Functioning of a third-generation MLV-packaging cell line (from [14])